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Author(s)	Lolin, YI; Sanderson, JE; Cheng, SK; Chan, CF; Pang, CP; Woo, KS; Masarei, JRL
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Hyperhomocysteinaemia and premature coronary artery disease in the Chinese

Y I Lolin, J E Sanderson, S K Cheng, C F Chan, C P Pang, K S Woo, J R L Masarei

Abstract

Objectives—To examine the prevalence of hyperhomocysteinaemia and compare it with the classic risk factors and vitamin status in Hong Kong Chinese patients with premature atherosclerotic coronary artery disease.

Design—Case-control study.

Setting—General hospital and community.

Subjects—Forty five patients (39 males) with significant coronary artery disease confirmed by angiography (32 post myocardial infarction) and 23 healthy volunteers (17 male), all aged less than 55 years.

Intervention—Standardised methionine-loading test.

Main outcome measures—Coronary artery disease, risk factors.

Results—More patients than controls had fasting hyperhomocysteinaemia (10/45 v 2/23, $P = 0.122$), post-methionine hyperhomocysteinaemia (17/45 v 1/23, $P = 0.008$), and an abnormal response to methionine (15/45 v 1/23, $P = 0.015$). A history of smoking was more frequent in patients (3/23 v 25/45, $P = 0.002$). Sixteen of 17 patients with hyperhomocysteinaemia but only nine of 28 with normohomocysteinaemia were smokers ($P = 0.0002$). Fasting plasma cholesterol concentrations (mean (SD)) were higher in hyperhomocysteinaemic patients (6.41 (1.58) mmol/l) than in controls (5.53 (0.90) mmol/l) ($P = 0.042$). Serum vitamin B-12 was not reduced and serum folate was higher in hyperhomocysteinaemic patients (35 (4) nmol/l) than normohomocysteinaemic patients (26 (9) nmol/l) ($P = 0.009$).

Conclusions—Although the prevalence of hyperhomocysteinaemia in Hong Kong Chinese is similar to that in white subjects, hyperhomocysteinaemia is not an independent risk factor for coronary artery disease and is associated with smoking. This may be of some consequence in view of the change to a more Western diet with more animal protein, and therefore methionine, coupled with a high frequency of cigarette smokers in this region. The causes of the hyperhomocysteinaemia are multifactorial but in this pilot study a deficiency of folate and/or vitamin B-12 did not seem to be one of them.

Keywords: hyperhomocysteinaemia; coronary artery disease; risk factors

Homocysteine is metabolised to cystathionine by pyridoxine dependent cystathionine- β -synthase and remethylated by folate dependent 5-methyltetrahydrofolate-homocysteine-methyl-transferase with vitamin B-12 as cofactor and by betaine homocysteine methyl-transferase¹⁻³ to methionine. Classic homocystinuria, due to deficiency of cystathionine- β -synthase, with a prevalence of up to 1:200 000 in white subjects, is associated with premature vascular disease^{2,4} as are other even rarer inborn errors of homocysteine metabolism.^{1,5}

In recent years mild to moderate hyperhomocysteinaemia has also been found to be associated with premature peripheral vascular,^{6,7} cerebrovascular,^{6,8} and coronary artery disease,^{9,11} and to be a risk factor independent of hyperlipidaemia, hypertension, or cigarette smoking.¹²⁻¹⁴ In white subjects the prevalence of this risk factor in those with premature vascular disease is about 30% greater than that in the general population.^{14,16} The causes of mild to moderate hyperhomocysteinaemia include genetic and dietary factors^{14,17,18} with enzyme disorders involved in metabolism to cystathionine,^{6,12,19,20} homocysteine remethylation,^{21,22} and cofactor deficiency^{23,25} being regarded as the most common.

The prevalence of, and mortality from, coronary artery disease in Chinese subjects is about one-eighth to one quarter of that in white subjects.²⁶ However, while the prevalence in the West is falling, in the Southern Chinese^{26,27} it is rising and this rise is attributed to the socioeconomic achievements of the past two decades²⁶ and a more Westernised lifestyle. Although recent studies confirm the importance of similar risk factors for coronary artery disease in Hong Kong as in the West,²⁷⁻³⁰ the prevalence of hyperhomocysteinaemia is unknown.

Therefore, we have investigated the prevalence of hyperhomocysteinaemia in Hong Kong Chinese with premature occlusive coronary artery disease and have compared it with the prevalence of the classic risk factors in order to ascertain its importance in this population.

Patients and methods

SUBJECTS

We studied 45 patients (39 males, six females), including 13 with ischaemic heart

Department of
Chemical Pathology,
The Chinese
University of Hong
Kong, Prince of Wales
Hospital, Hong Kong
Y I Lolin
S K Cheng
C F Chan
C P Pang
J R L Masarei

Department of
Medicine, The Chinese
University of Hong
Kong, Prince of Wales
Hospital, Hong Kong
J E Sanderson
K S Woo

Correspondence to:
Dr Y I Lolin, Department of
Chemical Pathology, The
Chinese University of Hong
Kong, Prince of Wales
Hospital, Shatin, Northern
Territory, Hong Kong.

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disease but no infarction and 32 with a history of myocardial infarction, and 23 apparently healthy controls (17 males and four females). All were less than 55 years of age (mean (SD), patients 48.2 (6.6) and controls 46.1 (7.3), $P = 0.192$). In all patients occlusive atherosclerotic coronary artery disease was confirmed by coronary angiography one week before methionine loading. The onset of symptoms in patients without infarction was up to two years before angiography. Patients with myocardial infarction were studied up to three years after the initial presentation. Myocardial infarction had been confirmed by serial electrocardiograms and changes in cardiac enzyme activity (serum creatine kinase, aspartate aminotransferase, and lactate dehydrogenase). Methionine loading was delayed until at least three months after the acute event. The controls were hospital staff and their friends and relatives. They were selected on the basis of their age, background, and lifestyle (reflecting similar diets and type of work (mostly white collar workers in the service and other light industries)) and amount of exercise to reflect the characteristics of the patients and this population generally. The exclusion criteria were overt chronic disease including renal and liver insufficiency; acute illness or surgery in the previous three months; alcohol abuse; medication with drugs inducing liver enzymes, lipid lowering drugs, or vitamins; and pregnancy. The study was approved by the institutional human medical ethics committee and informed consent was obtained from all subjects. The procedures followed accorded with institutional guidelines.

METHIONINE LOADING AND BLOOD SAMPLES OBTAINED

The methionine-loading test with 0.1 g/kg body weight L-methionine (Scientific Hospital Supplies, Liverpool) was performed as previously described.^{6,12} Blood samples were taken for fasting and six hour post-methionine homocysteine; for a full range of laboratory tests to exclude covert disease; and for fasting plasma lipids, serum vitamin B-12, and serum and red blood cell folate concentrations. During the six hours before we took the post-methionine blood sample the subjects were allowed a protein-free diet but they found it unpalatable and continued fasting until the end of the study.

RISK FACTORS

Hypertension was diagnosed if it was recorded in the patients' medical notes and, in both groups, if the supine systolic pressure was above 160 mm Hg and/or the diastolic above 90 mm Hg on at least two occasions. Hyperlipidaemia was diagnosed if there was a record in the patients' medical notes of fasting plasma cholesterol and/or triglyceride concentrations above the recommended laboratory reference range in the Hong Kong Chinese population (5.2 mmol/l and 1.8 mmol/l, respectively), or if these concentrations were recorded in both groups during the study. We

used the results obtained at the time of methionine loading to compare the fasting plasma lipid concentrations and their relation to plasma homocysteine concentrations in both groups. However, to classify patients as hyperlipidaemic or normolipidaemic, we took into account the plasma lipid concentrations in the patients' clinical records, particularly in those on dietary lipid restriction where the results before dietary intervention were used (patients on lipid-lowering drugs had been excluded from the study). Although plasma low density lipoprotein (LDL), high density lipoprotein (HDL), and HDL cholesterol were also measured during methionine loading they are not included because in most cases they were not assayed when the patient originally presented. Smokers included current cigarette smokers or those who had stopped smoking within six months of the start of the study. The smoking status at the time of initial diagnosis and, in ex-smokers, the length of abstinence from smoking were recorded and will be used in follow up studies. No subject smoked cigarettes on the day of methionine loading, and none was a pipe or cigar smoker.

HOMOCYSTEINE ANALYSIS AND DEFINITION OF HYPERHOMOCYSTEINAEMIA

After venesection, blood was immediately put into a heparinised bottle on ice, protected from sunlight, and centrifuged within 10 minutes. Plasma was stored at -70°C until analysis within six months. The method for total plasma homocysteine measurement was based on those of Ubbink *et al*³⁰ and Jacobsen *et al*,³¹ using sodium borohydride for reduction and dis-association of the amino acid from proteins, trichloroacetic acid for protein precipitation, and SBD-F (ammonium 7-fluorobenzo-2-oxa-1,3 diazole-4-sulphonate) for derivatisation of the supernatant and N-[2 mercaptopropionyl] glycine (Sigma, St Louis, USA) and as an internal standard. Homocysteine was measured by isocratic reverse phase high performance liquid chromatography on a Hewlett Packard HP1090 High Performance Liquid Chromatograph fitted with a HP 1049A fluorescence detector.

The assay was linear to a plasma homocysteine concentration of at least 100 $\mu\text{mol/l}$. The recoveries for homocysteine added to plasma to obtain concentrations of 20, 60, and 80 $\mu\text{mol/l}$ were 103, 91, and 110% respectively. The within-batch imprecision (CV%) ($n = 16$ for each) was 7.8%, 6.9%, and 3.7% at plasma homocysteine concentrations of 20, 60, and 80 $\mu\text{mol/l}$ respectively. The corresponding between-batch imprecision ($n = 16$ for each) was 9.3%, 10.6%, and 5.2%.

As in previous studies^{6,12} fasting and post-methionine hyperhomocysteinaemia and methionine intolerance (the rise in plasma homocysteine from the fasting concentration after methionine loading) were considered to be present if plasma homocysteine was above the respective adjusted control mean + 2SD. The adjusted control reference range was obtained after removing subjects with values higher than the control mean + 3SD from the

Table 1 Fasting and post-methionine load plasma homocysteine concentrations, the increase in homocysteine from the fasting concentration after the load, and the prevalence of hyperhomocysteinaemia in controls and patients (geometric means are shown in *italics*)

	Homocysteine concentration ($\mu\text{mol/l}$)			Prevalence \ddagger		
	Fasting (mean (SD))	Post-load (mean (SD))	Increase (mean (SD))	Fasting hyperhomocysteinaemia	Post-methionine hyperhomocysteinaemia	Pathological response to methionine load
Control (n = 23)*	12.1 (4.7)	29.1 (9.2)	17.0 (6.3)	1 (4.3%)	1 (4.3%)	1 (4.3%)
Control (n = 21)†	11.4 (1.4)	27.7 (1.4)	15.8 (1.5)	1 (4.8%)	0	0
Control (n = 21)‡	11.3 (3.6)	27.4 (7.6)	16.1 (5.3)	1 (4.8%)	0	0
Patient (n = 45)	10.5 (1.7)	25.1 (1.2)	14.2 (1.3)	10 (22.2%)	17 (37.8%) (d)	15 (33.3%) (e)
	14.9 (4.4) (a)	39.1 (16.3) (b)	24.7 (14.5) (c)			
	14.3 (1.4) (f)	37.0 (1.5) (g)	21.8 (1.6) (h)			

*Including all control data.
†Without the two control outliers, one with fasting and one with post-methionine homocysteine concentration above the overall control mean + 3 (SD).
‡Calculated using all subjects but with the control mean recalculated after removing the two outliers with homocysteine concentrations above mean + 3 (SD).
(a) P = 0.004 for patients v 23 controls, P = 0.0008 for patients v 21 controls; (b) P = 0.003 for patients v 23, P = 0.002 for patients v 21 controls; (c) P = 0.01 for patients v 23 controls, P = 0.004 for patients v 21 controls, Mann-Whitney test; (d) P = 0.008 for patients v 23 controls, P = 0.005 for patients v 21 controls; (e) P = 0.015 for patients v 23 controls, P = 0.005 for patients v 21 controls, Fisher's exact test; (f) P = 0.007 for patients v 23 controls, P = 0.0001 for patients v 21 controls; (g) P = 0.003 for patient v 23 controls, P = 0.0002 for patients v 21 controls; (h) P = 0.009 for patient v 23 controls, P = 0.0004 for patients v 21 controls, Student's *t* test.

original, overall control data and recalculating the mean (and SD).

STATISTICAL ANALYSIS
The arithmetic means of the fasting, post-methionine homocysteine concentrations, the response to methionine, and other variables between the groups were compared using the Mann-Whitney test and one-way ANOVA. Because the plasma homocysteine concentrations showed a positively skewed distribution, geometric means were also compared, using the independent Student's *t* test. The correlations between fasting plasma homocysteine, vitamin B-12, serum and red blood cell folate and between fasting plasma homocysteine and fasting lipids were examined using Pearson's correlation coefficients and stepwise linear regression. The prevalence of risk factors in the two groups was compared by Fisher's exact test. To assess whether hyperhomocysteinaemia was associated with coronary artery disease independently of the confounding factors smoking, hyperlipidaemia, and hypertension we used stepwise logistic multivariate regression analysis with coronary artery disease as a binary outcome variable and logarithms of post-methionine plasma homocysteine and presence or absence of high plasma cholesterol and triglyceride concentrations, smoking, and hypertension as independent variables. The statistical analyses were performed using an Abstat software package except for the comparisons of the geometric means of the plasma homocysteine concentrations and the logistic multivariate analysis, both of which were done using a BMDP/Dynamic statistics software package (BMDP/Dynamic Release 7.0, (C) 1993, BMDP Statistics Software, Los Angeles, USA). The level of statistical significance was P < 0.05.

Results
HYPERHOMOCYSTEINAEMIA
One control had a fasting and another post-methionine homocysteine concentrations greater than the overall control arithmetic mean + 3SD. After removal of these subjects and recalculation of the arithmetic mean there was still one control with fasting hyperhomocysteinaemia but none with post-methionine hyperhomocysteinaemia or abnormal tolerance to homocysteine. The fasting and post-methionine plasma homocysteine concentrations and the rise in homocysteine after methionine were all significantly higher in patients than controls both with (P = 0.0008, P = 0.002, P = 0.01, respectively) and without (P = 0.004, P = 0.003, P = 0.004, respectively) removing the control outliers from the original data (table 1). Similar results were obtained with geometric mean homocysteine concentrations (table 1). Fasting hyperhomocysteinaemia (table 1) was nearly three times more frequent in patients than controls (10 out of 45 v 1 out of 23) but the prevalence was not statistically different between the groups (P = 0.122), possibly because the numbers were small. Post-methionine hyperhomocysteinaemia and an abnormal response to methionine (table 1) were both significantly more frequent in patients (17 out of 45 v 1 out of 23, P = 0.008, and 15 out of 45 v 1 out of 23, P = 0.015, respectively). Eight patients had normal fasting but increased post-methionine plasma homocysteine concentrations. One patient had raised fasting and post-methionine plasma homocysteine concentrations but the difference between the two was normal (normal response to methionine).

Table 2 Plasma concentration of vitamin B-12 and folate and of red blood cell folate in controls and patients with and without fasting hyperhomocysteinaemia

Variable	Controls	Patients		
		All	With	Without
Number	23	45	10†	35
B-12 (pg/ml (SD))	339 (86)	378 (159)	378 (139)	312 (121)
Red blood cell folate (nmol/l (SD))	538 (273)	675 (281)	618 (275)	656 (229)
Serum folate (nmol/l (SD))	22 (10)	30 (11)	35 (4)*	26 (9)

*P = 0.026 (ANOVA), P = 0.009 (Mann-Whitney) for patients with hyperhomocysteinaemia v those without hyperhomocysteinaemia.
†Multiple linear regression: homocysteine v B-12, r = 0.214, P = 0.359; + v RBC folate, r = 0.490, P = 0.072; + v serum folate, r = 0.975, P = 0.022.

**RELATION BETWEEN
HYPERHOMOCYSTEINAEMIA AND VITAMIN B-12,
FOLATE, AND RED BLOOD CELL FOLATE**
Table 2 shows serum vitamin B-12, serum folate, and red blood cell folate concentrations in controls and patients, and patients with and without fasting hyperhomocysteinaemia. Serum

Table 3 Frequency distribution of hyperlipidaemia, hypertension, smoking, and gender in controls and patients with and without post-methionine hyperhomocysteinaemia and the mean fasting blood lipids on the day of the study

Variable	Controls		Patients		Patients with vascular disease			
					Hyperhomocysteinaemia		Normohomocysteinaemia	
	No/total	%	No/total	%	No/total	%	No/total	%
Hypertension diastolic	5/23	21.7	11/45	24.4	6/17	35.3	5/28	17.9
Hypertension systolic	0/23	0	9/45	20.0	5/17	29.4	4/28	14.3
Hypertriglyceridaemia	3/23	13.0	10/45	22.2	6/17	35.3	4/28	14.3
Fasting plasma triglycerides mmol/l (SD)	1.27 (0.71)	—	1.50 (0.87)	—	1.26 (0.57)	—	1.56 (0.94)	—
Hypercholesterolaemia	13/23	56.5	25/45	55.6	11/17	64.7	14/28	50.0
Fasting plasma cholesterol mmol/l (SD)	5.53 (0.90)	—	5.79 (1.25)	—	6.41 (1.58)*	—	5.60 (1.06)	—
Smoking	3/23	13.0	25/45	55.6†	16/17	94.1	9/28	32.1‡
Sex (M)	17/23	73.9	39/45	86.7	16/17	94.1	23/28	82.1

*P = 0.042 for patients with hyperhomocysteinaemia v controls (Mann-Whitney).
†P = 0.002 for patients v controls (Fisher's exact test).
‡P = 0.0002 for patients with v patients without hyperhomocysteinaemia (Fisher's exact test).

folate was higher, though non-significantly, in patients than in controls (P = 0.103) and also in hyperhomocysteinaemic patients than in controls (P = 0.092). It was, however, significantly higher in patients with than in those without fasting hyperhomocysteinaemia (P = 0.026 by Anova, P = 0.009 with the Mann-Whitney test). There were no differences in serum vitamin B-12, serum folate, and red blood cell folate concentrations between patients with (368 (101) nmol/l, 29 (13) nmol/l, 593 (223) nmol/l, respectively) and without post-load hyperhomocysteinaemia (311 (128) pg/l, 26 (10) nmol/l, 686 (251) nmol/l, respectively) or between patients with a normal (322 (127) pg/l, 25 (9) nmol/l, 680 (240) nmol/l, respectively) and abnormal methionine tolerance (351 (108) pg/l, 28 (11) nmol/l, 587 (253) nmol/l). Further, on comparison of the correlations between concentrations of homocysteine and vitamins in the various groups, there was a trend towards a positive correlation between fasting homocysteine and serum folate concentrations in patients with fasting hyperhomocysteinaemia (r = 0.779, P = 0.068) and a positive correlation with multiple linear regression using fasting homocysteine as the dependant variable and adding first vitamin B-12 (r = 0.2139, P = 0.359), followed by red blood cell folate (r = 0.490, P = 0.72), and then serum folate (r = 0.975, P = 0.022). This was not observed with the other groups.

No subject had a reduced serum vitamin B-12 concentration. One control with normohomocysteinaemia had a reduced serum folate concentration. Eight controls (34.8%) and five patients (11.1%), with no significant difference in the distribution between the main groups or between those with and without hyperhomocysteinaemia, had low red blood cell folate.

HYPERHOMOCYSTEINAEMIA AND CONVENTIONAL RISKS FOR CORONARY ARTERY DISEASE
Table 3 shows the frequency distribution of hyperlipidaemia, hypertension, and cigarette smoking in controls and patients with post-methionine hyperhomocysteinaemia, and the mean fasting plasma cholesterol and triglyceride concentrations. A history of cigarette smoking was the only risk factor that was significantly more frequent in patients (P = 0.002). Further, a history of smoking was obtained in all but one patient with hyperhomocys-

teinaemia and in only a third of those without (P = 0.0002). All the smokers were male. When we removed the female subjects from the groups the higher prevalence of smoking in patients with hyperhomocysteinaemia remained significant (P = 0.0004). There was no difference in fasting plasma lipids between the groups when we used Anova, but with the Mann-Whitney test plasma cholesterol was higher in patients with hyperhomocysteinaemia than in controls (P = 0.042). No correlation was obtained between plasma homocysteine and plasma lipids in either group when we used simple regression or stepwise multiple linear regression. However, with stepwise logistic regression, coronary artery disease as a binary outcome, and hyperhomocysteinaemia and other risk factors as independent variables, only smoking was found to be a significant independent variable (for smoking P = 0.0004, for plasma homocysteine concentrations P = 0.1394, for the blood pressure P = 0.9224, for plasma cholesterol concentration P = 0.4140, for plasma triglyceride concentrations P = 0.2144).

Discussion

Since the first report, in 1976³³ of an association between mild hyperhomocysteinaemia and premature coronary artery disease, accumulated reports to date have shown a prevalence of mild to moderate homocysteinaemia in 12% to 40% of patients with premature vascular disease.¹⁴⁻¹⁸ This prevalence seems to vary with the anatomical site and has been reported to be highest in cerebrovascular and lowest in coronary artery disease.¹⁴

Studies in white subjects have shown fasting hyperhomocysteinaemia in 11% to 24%⁹⁻¹⁰⁻¹³ and post-methionine hyperhomocysteinaemia in 14% to 30%¹¹⁻¹²⁻³⁴ of subjects with premature coronary vascular disease. Our findings suggest that hyperhomocysteinaemia may be at least as common in Hong Kong Chinese patients. The plasma homocysteine concentrations were also similar to those reported in white subjects.⁹⁻²²⁻³⁵ Although this is the first report on hyperhomocysteinaemia in a Chinese population, increased homocysteine concentrations have also been noted in Japanese patients with vascular disease.³⁶⁻³⁷

Mild to moderate hyperhomocysteinaemia is

associated with both genetic and nutritional factors. In one study post-methionine homocysteine concentrations were comparable to those in subjects with obligate heterozygosity for cystathionine- β -synthase in 18 (30%) out of 60 patients with coronary artery disease.¹² Others have noted reduced concentrations of cystathionine- β -synthase in most of their patients with premature vascular disease and hyperhomocysteinaemia.¹⁷ A thermolabile variant of methylenetetrahydrofolate reductase, with 50% of the activity of the normal enzyme, was noted in about 8% of patients without, 13% with moderate, and 18% of those with severe coronary artery occlusion.²² In the general population the prevalence of heterozygosity for cystathionine- β -synthase is up to about 1% and that of thermolabile methylenetetrahydrofolate reductase up to about 5%,²¹ suggesting that these enzymes may be a major cause of genetic hyperhomocysteinaemia. Genetic factors influencing homocysteine metabolism may also be involved in about 10% of cases of early familial coronary artery disease.⁹ Nutritional deficiency of vitamins B-12 and B-6 and, particularly, folate may be even more important. Blood folate^{3, 12, 13} and, less frequently, blood vitamin B-12^{3, 12, 24} and B-6^{11, 13, 35} concentrations have been shown to be inversely related to basal plasma homocysteine concentrations. Further, vitamin treatment normalises homocysteine concentrations,^{17, 38, 39} though it is not yet known if this also reduces the risk of coronary artery disease.^{13, 23} However, nutritional folate deficiency is thought to be present in up to 40% of the US population and prophylaxis with increased dietary intake of folate has already been suggested.²³

Methionine loading has been often used to unmask methionine intolerance in patients with normal fasting plasma homocysteine concentration.¹¹ It has also been used to distinguish between causes of hyperhomocysteinaemia. Though fasting hyperhomocysteinaemia has been reported in association with vitamin deficiencies,^{14, 32} with the heat labile form of methylenetetrahydrofolate reductase,²² and with post-methionine hyperhomocysteinaemia caused by heterozygous cystathionine- β -synthase,^{3, 6, 7, 12} these distinctions are not clear-cut. Subnormal activity of cystathionine- β -synthase can be caused by vitamin B-6 deficiency alone.³⁸ The co-existence of heterozygosity for the enzyme and deficiency of vitamins B-6 and B-12 and/or folate has been reported.^{11, 12, 17} Methionine intolerance associated with normal activity of cystathionine- β -synthase but with folate deficiency has also been noted.¹² In a recent study in patients with post-methionine hyperhomocysteinaemia and with normal blood concentrations of B-6, B-12, and folate, in most the response to methionine loading became normal after treatment with vitamin supplements.³⁸ This suggested that the patients who responded to B-6 might have been heterozygous for cystathionine- β -synthase whereas the non-responders might have had the heat labile form of methylenetetrahydrofolate reductase.

We did not assay the activities of cystathion-

ine- β -synthase or thermolabile methylenetetrahydrofolate reductase, or measure blood concentrations of B-6. Thus the predominant cause(s) of hyperhomocysteinaemia in our patients is unknown. Further, the study was limited by the number of subjects, particularly controls. Premature coronary vascular disease is relatively rare in the Hong Kong Chinese. In addition, recruiting volunteers for the study, especially for methionine loading, was difficult in this community because it entailed a prolonged fast because the subjects found the protein free meals unpalatable. However, the results of this study differed from most previous reports in two major aspects. Firstly we noted higher plasma folate concentrations in hyperhomocysteinaemic than normocysteinaemic subjects as well as a positive correlation between plasma folate and fasting homocysteine concentrations in those who were hyperhomocysteinaemic. This was unexpected and contradicted previous reports.^{3, 12, 13} The patients denied being on vitamin supplements. Although in this community green, leafy, lightly steamed vegetables (for example, broccoli, Chinese white and flowering cabbage, Chinese kale, Chinese asparagus) are commonly eaten throughout the year (probably more than in the West, personal observation) and folate deficiency is generally rare, the concentrations should have been similarly high in all groups. A possible explanation for these findings may be that these patients have a generally higher intake of green vegetables, meat, and animal fat than other subjects in the study, since they also had higher fasting plasma cholesterol concentrations and also higher plasma B12 concentrations (though this difference was not significant). This is being investigated and requires confirmation with larger subject numbers. The prevalence of the thermolabile methyltetrahydrofolate reductase is also being examined in this population. Secondly, we noted a strong correlation between hyperhomocysteinaemia, smoking, and coronary artery disease. However, only smoking was found to be an independent risk factor, suggesting that the association between hyperhomocysteinaemia and coronary artery disease was likely to be the result of the higher prevalence of smokers in the hyperhomocysteinaemic group in this cohort or an interaction between the two risk factors. This needs further study. Previously, hyperhomocysteinaemia was thought to be an independent risk factor for vascular disease.^{12, 13} Although the association with smoking in this study could be coincidental because the numbers of subjects were small, it was also recently seen in white subjects with hyperhomocysteinaemia and occlusive vascular disease.^{35, 40} A weak association with hypercholesterolaemia had also been noted by some but not other workers.⁴¹

Classic coronary artery disease risk factors are as important in the Hong Kong Chinese as in the Western population, although at present hypertension and cigarette smoking may be more important.^{27, 29} Whereas the incidence of coronary artery disease and myocardial infarction is falling in the West generally and more

particularly in the higher socioeconomic classes, in the Southern Chinese the trend is in the opposite direction.^{26,27} Increased affluence, a change to a more sedentary Western lifestyle, to a diet richer in animal and dairy products, particularly in the younger generations, and cigarette smoking are thought the main reasons.²⁶ Proteins of animal origin contain two to three times more methionine than proteins of plant origin. The aetiology of hyperhomocysteinaemia is multifactorial but the results of this study suggest that in this population vitamin deficiency is unlikely to be a cause. Defective utilisation of folate is possible but genetic disorders involved in homocysteine metabolism, already shown to be common in white subjects, are more likely. While heterozygosity for homocystinuria caused by cystathionine synthase deficiency or the presence of the thermolabile methylenetetrahydrofolate reductase as a risk factor should remain constant, it may not have been of significance in this population until compounded with a high methionine diet and, possibly, smoking.

In conclusion, the results of this study, although in a small number of subjects, suggest that the prevalence of hyperhomocysteinaemia in the Hong Kong Chinese with coronary artery disease is similar to, and possibly higher, than in the West. Absence of overt vitamin B-12 and folate deficiency and the presence of high folate concentrations in hyperhomocysteinaemic patients suggest that vitamin deficiency was unlikely to be of major aetiological importance. The cause for the higher folate concentrations in hyperhomocysteinaemic patients is being investigated. An association between hyperhomocysteinaemia and smoking was also noted with only smoking being an independent variable for coronary vascular disease. This needs further evaluation.

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